RESEARCH ARTICLE

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Decoding Molecular Interactions: Unraveling the Crosstalk between the Wnt Pathway and Key Signaling Networks by miRNA in Colorectal Cancer Progression

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Abstract

Background: Colorectal cancer (CRC) is intricately influenced by dysregulated microRNAs (miRNAs) targeting the Wnt signaling pathway, a phenomenon pivotal in CRC initiation and progression. The exploration of miRNA-Wnt interactions holds promise for innovative therapeutic strategies in CRC treatment. **Methods:** a comprehensive list of genes influenced by dysregulated miRNAs targeting the Wnt pathway was compiled. High-scoring genes from the miRDB database underwent further analysis. Protein-protein interaction networks were constructed using Cytoscape and StringApp 2.0, with hub proteins identified through MCC, MNC, DMNC, and Degree algorithms. Gene ontology, KEGG enrichment analysis, CytoCluster, and promoter motif analysis were employed to characterize gene functions, associations, dysregulated clusters, and regulatory elements. **Results:** Protein-protein interaction networks unveiled 15 central hub proteins, including EP300, NRAS, NF1, CCND1, SMAD4, SOCS7, SOCS6, NECAP1, MBTD1, ACVR1C, ESR1, CREBBP, and PIK3CA. Gene ontology and KEGG analysis revealed their involvement in critical biological processes, cellular components, and molecular functions. CytoCluster analysis identified dysregulated miRNA-targeted gene clusters linked to cancer-related pathways. Promoter motif analysis provided insights into regulatory elements governing hub protein expression. **Conclusion:** The identified hub proteins, enriched in cancer-related pathways, offer potential therapeutic targets. These findings pave the way for future research, enhancing our ability to develop targeted interventions for improved outcomes in CRC treatment.

Keywords: Colorectal cancer- Wnt signaling- systems biology- promotor motif analysis- subnetwork analysis

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Introduction

The importance of gene networks in the initiation and progression of colorectal cancer (CRC) lies in their role as intricate regulatory systems that govern the complex molecular processes underpinning tumorigenesis. Dysregulation within these networks can disrupt critical signaling pathways, leading to uncontrolled cell growth, evasion of apoptosis, and invasive behavior characteristic of CRC The importance of gene networks in the initiation and progression of colorectal cancer (CRC) lies in their role as intricate regulatory systems that govern the complex molecular processes underpinning tumorigenesis. Dysregulation within these networks can disrupt critical signaling pathways, leading to uncontrolled cell growth, evasion of apoptosis, and invasive behavior characteristic of CRC [1]. By examining the interconnectedness of genes and proteins, researchers can pinpoint central hub proteins and dysregulated gene clusters that serve as key drivers in CRC development. Understanding these gene networks provides a holistic view of the molecular landscape, offering insights into potential therapeutic targets and diagnostic markers [2]. Unraveling the dynamics of gene networks in CRC is essential for advancing our comprehension of the disease and fostering the development of targeted interventions, paving the way for more effective treatments and improved patient outcomes.

The Wnt signaling pathway holds paramount importance in both the initiation and progression of colorectal cancer (CRC), as its dysregulation significantly contributes to tumorigenesis. Aberrant activation of the Wnt pathway, often driven by mutations in key genes such as APC and β-catenin, promotes uncontrolled cell proliferation and inhibits apoptosis, hallmark features of CRC [3]. Beyond its individual impact, the Wnt pathway

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engages in intricate crosstalk with other cellular pathways, amplifying its influence on CRC progression. Notably, its cross-interactions with the MAPK and PI3K-Akt pathways contribute to the intricate molecular network governing CRC. The synergistic effects of this crosstalk further underscore the Wnt pathway's central role in orchestrating the complex signaling cascades involved in CRC development, offering potential avenues for targeted therapeutic interventions aimed at disrupting these interconnected pathways and impeding colorectal cancer progression [4].

Small RNA molecules with pivotal roles in post-transcriptional gene regulation, have emerged as key players in the pathogenesis of CRC [5]. Specifically, miRNAs targeting the Wnt signaling pathway, a fundamental cellular pathway implicated in CRC, have garnered significant attention. Dysregulation of these miRNAs can profoundly influence critical cellular processes, including proliferation, differentiation, and apoptosis, contributing to the initiation and progression of CRC [6]. While the importance of miRNA-Wnt interactions in CRC is recognized, a comprehensive investigation into the functional implications of these interactions, particularly through the identification of hub proteins and dysregulated gene clusters, is essential [7]. This study aims to unravel the complex interplay between miRNAs and the Wnt pathway in CRC, employing an integrative approach that includes protein-protein interaction networks, gene ontology, KEGG enrichment analysis, CytoCluster analysis, and promoter motif analysis. By elucidating the molecular landscape of CRC at a systems level, this research seeks to provide valuable insights into potential therapeutic targets and pave the way for the development of precision treatments tailored to the unique molecular signatures of colorectal cancer patients. By examining the interconnectedness of genes and proteins, researchers can pinpoint central hub proteins and dysregulated gene clusters that serve as key drivers in CRC development. Understanding these gene networks provides a holistic view of the molecular landscape, offering insights into potential therapeutic targets and diagnostic markers. Unraveling the dynamics of gene networks in CRC is essential for advancing our comprehension of the disease and fostering the development of targeted interventions, paving the way for more effective treatments and improved patient outcomes.

Materials and Methods

Data Collection and Protein-Protein Interaction (PPI) Network Construction

To investigate the role of miRNAs in colorectal cancer through the Wnt signaling pathway, a comprehensive list of miRNAs was obtained from the 2022 reviwe article [8]. This study identified miRNAs that modulate components of the Wnt pathway in colorectal cancer. The identified miRNAs were used to extract their target genes from the miRDB database (https://mirdb.org), selecting interactions with a confidence score >95. This process yielded 921 genes targeted by dysregulated miRNAs [9].

Protein-protein interactions were predicted using the STRING database (version 11.5) [10]. The analysis incorporated both direct (physical) and indirect (functional) associations, derived from computational predictions, experimental data, and aggregated interactions from other databases. The resulting network was visualized and analyzed using Cytoscape (version 3.9.1), an open-source platform for biological network visualization. CytoHubba, a plugin for Cytoscape, was used to identify hub proteins within the network. Hub proteins were determined using four topological algorithms: MCC, MNC, DMNC and Degree. The analysis identified hub genes by ranking proteins based on their centrality scores. The top-ranking hub genes, crucial for network connectivity and implicated in the Wnt pathway, were selected for further analysis [11].

Gene Ontology (GO) and KEGG Pathway Enrichment Analysis

Functional annotation of proteins targeted by miRNAs was performed to uncover their biological significance in colorectal cancer progression. The analysis utilized EnrichR database to conduct Gene Ontology (GO) and Wiki Pathway enrichment studies. GO terms were categorized into three main domains: Biological Processes (BP): Processes such as transcription regulation, cell differentiation, and apoptosis were identified to have significant enrichment among the targeted proteins. Molecular Functions (MF): Functions including protein binding, kinase activity, and regulatory roles were highlighted. And Cellular Components (CC): Identified components were associated with intracellular organelles, membranes, and the extracellular matrix [12].

Network Cluster Analysis Using CytoCluster

The CytoCluster plugin (version 2.1.0) in Cytoscape (version 3.9.1) was utilized to analyze the protein-protein interaction (PPI) network of miRNA-targeted genes. The one-cluster algorithm was applied to identify highly interconnected subgraphs that represent significant protein complexes within the network. : One-cluster algorithm was selected for its efficiency in detecting cohesive submodules within the network [13].

Transcription factor perturbation and promoter analysis of hub genes

To explore transcription factors (TFs) influencing target genes, the ENCODE and ChEA Consensus TFs dataset from the Enrichr platform was utilized. This analysis identified key transcription factors whose perturbations might regulate the expression of hub genes. The results were ranked based on their combined scores, highlighting the most significant TFs associated with the hub genes [14].

For promoter Motif Analysis the promoter regions of the identified hub genes were analyzed to uncover conserved motifs and regulatory elements. Upstream flanking regions (UFRs) spanning 1 kilobase pair (1 kbp) from the transcription start site of hub genes were obtained from the Ensembl database. Tomtom (from the MEME Suite) was employed to identify motifs within the promoter sequences. The analysis focused on motifs with: P-value < 0.001 and E-value < 0.1. To predict potential

biological roles of the identified motifs, the GOMO tool (MEME Suite) was utilized. Functional annotations were mapped to the associated biological processes, molecular functions, and cellular components [15].

Drug Bank Analysis: Identification of Interacting Drugs and Compounds

The DrugBank database was used to identify drugs or experimental compounds, or natural substances that could interact with the hub proteins identified in the study, providing insights into potential therapeutic interventions for colorectal cancer [16].

Results

Protein-Protein Interaction (PPI) and Hub Protein Network of Targeted Genes

In the first step of this study, a list of dysregulated microRNAs (miRNAs) in colorectal cancer, specifically targeting the Wnt/β-catenin signaling pathway, was identified[8]. As shown in Table 1, miRNAs such as miR-27a, miR-590-3p, and miR-130a were among the key players targeting critical genes in this pathway, including SFRP1, WIF1, and NKD2, respectively.

The PPI network constructed using the STRING database comprised interactions among the 921 genes targeted by dysregulated miRNAs. Figure 1A represents the full interaction network, showcasing a dense network of protein interactions. By applying CytoHubba's ranking algorithms (MCC, DMNC, Degree, MNC), hub proteins were identified based on their centrality scores. As depicted in Figure 1B, the hub proteins, highlighted in green, play a crucial role in maintaining network connectivity [17].

The top hub proteins, listed in Table 2, include: EP300, a critical co-activator in transcriptional regulation [18]. NRAS, involved in signaling pathways that regulate cell growth and differentiation [19]. NF1, a tumor suppressor known for its role in Ras signaling [20]. These proteins were found to be essential nodes within the network and are implicated in key processes of colorectal cancer progression.

Functional Enrichment Analysis of Targeted Genes

To uncover the biological relevance of the miRNA-targeted genes in colorectal cancer, a functional enrichment analysis was performed. The results were

Table 1. miRNAs Regulate the Pathogenesis of Colorectal Cancer through Targeting the Specific Components of the Wnt/β-catenin Signaling Pathway

MicroRNA	Gene target(s)
miR-27a	*SFRP1*
miR-27-3p	*RXRα*
miR-552	*DACH1*
miR-103/107	*AXIN2*
miR-183-5p	*RCN2*
TrkC-miR2	*APC2*
miR-130a	*NKD2*
miR-942	*APC*
miR-590-3p	*WIF1, DKK1*
miR-217	*DKK1*
miR-410	*DKK1*
miR-574-5p	*QKI6/7*
miR-452	*GSK3β*
miR-224	*GSK3β, SFRP2, BTRC*
miR-501-3p	*APC*
miR-103a/miR-1827	*APC, APC2*
miR-31-5p	*LATS2*
miR-19a-3p	*FOXF2*
miR-425-5p	*CTNND1*
miR-346-5p	*FBXL2*

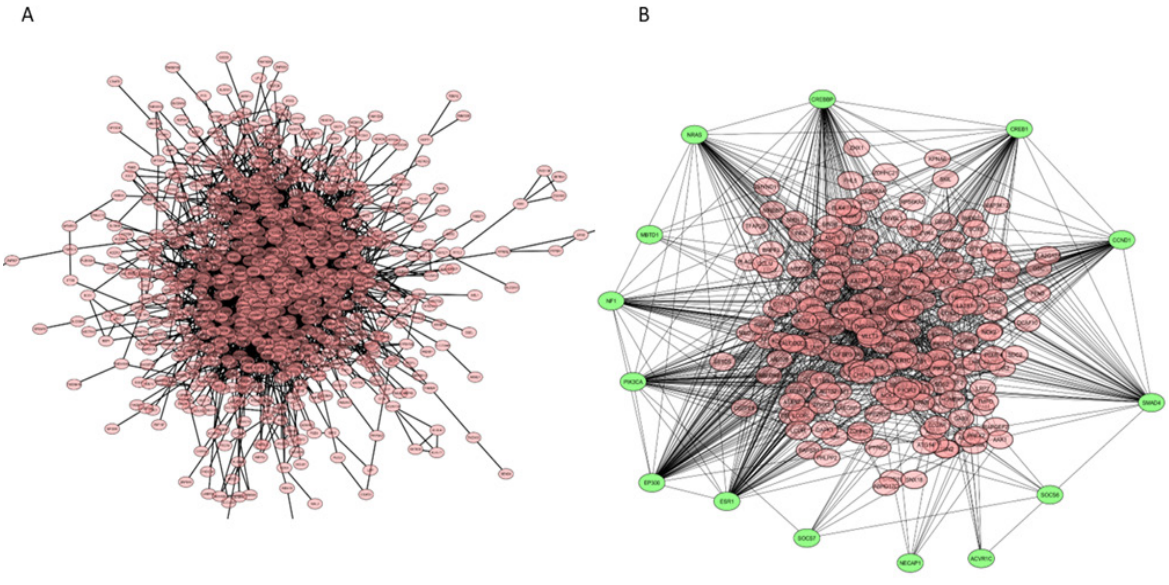

Figure 1. A: all proteins targeted by dysregulated miRNA. B: subnetwork analysis of hub proteins; hub proteins are highlighted in green

Table 2. Hob Proteins in Genes Targeted by Dysregulated miRNA

Name	Score	Algorithm	Rank
EP300	3004752	MCC/Degree/MNC	1,1,1
NRAS	2898336	MCC	2
NF1	2895635	MCC	3
CCND1	2822323	MCC	4
SMAD4	2764993	MCC	5
SOCS7	0.768339	DMNC	1
SOCS6	0.768339	DMNC	1
NECAP1	0.713237	DMNC	3
MBTD1	0.713237	DMNC	3
ACVR1C	0.695163	DMNC	5
ESR1	72	Degree/MNC	2,2
CREBBP	61	Degree/MNC	3,3
PIK3CA	58	Degree/MNC	4,4
CREB1	57	Degree/MNC	5,5

categorized into three main domains of Gene Ontology (GO) and WikiPathway analyses: Cellular Components (CC) identified several subcellular locations associated with the miRNA-targeted proteins, including: Intracellular Membrane-Bounded Organelles (GO:0043231): This category was significantly enriched, indicating the involvement of these proteins in cellular compartments critical for signaling and metabolic processes. Nucleus (GO:0005634): Highlighting roles in gene regulation and transcriptional activities. Cell-cell junctions (GO:0005911) and membrane-associated regions were also enriched, emphasizing the potential roles of these proteins in cell communication and signaling. The molecular functions (MF) enriched among the targeted proteins included: GTPase Regulator Activity (GO:0030695): Suggesting a role in controlling intracellular signaling cascades. Protein Kinase Regulator Activity (GO:0019887): Indicating the involvement in modulating kinase activities, which are central to cell growth and division. Ligand-dependent Nuclear Receptor Binding: Highlighting the interaction

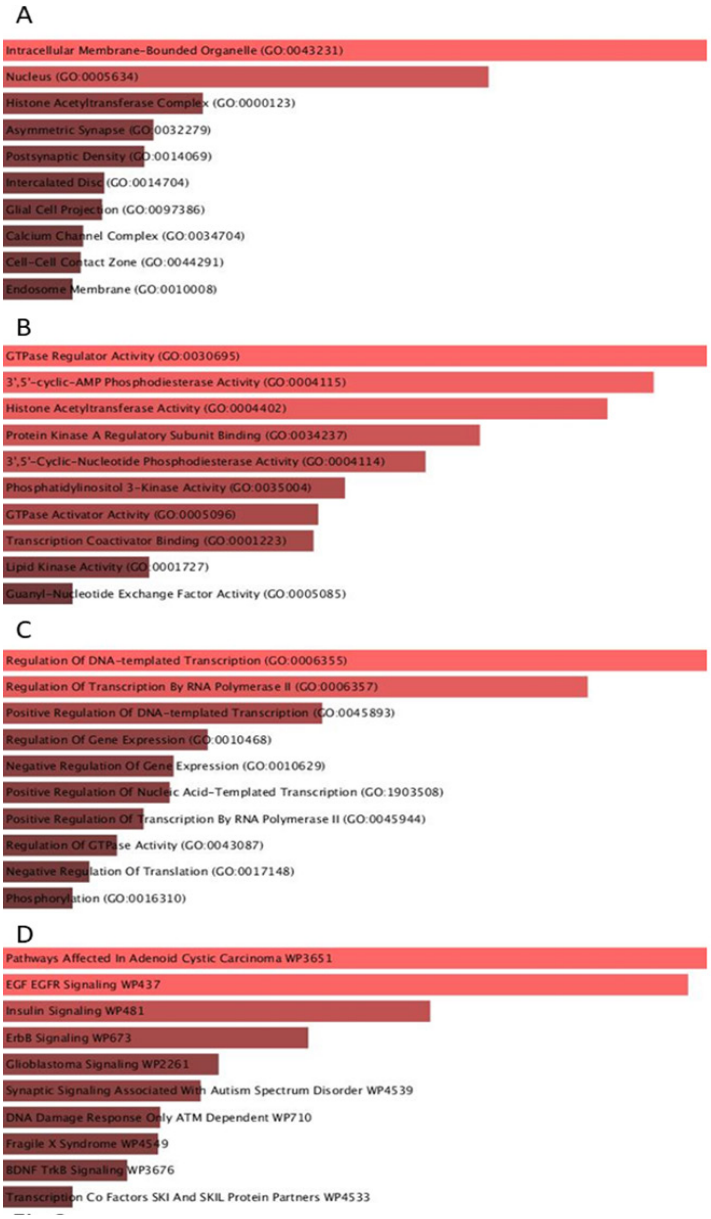

Figure 2. A: CC, B: M ,C: BP, D: Wiki pathway analysis 2024, of all proteins targeted

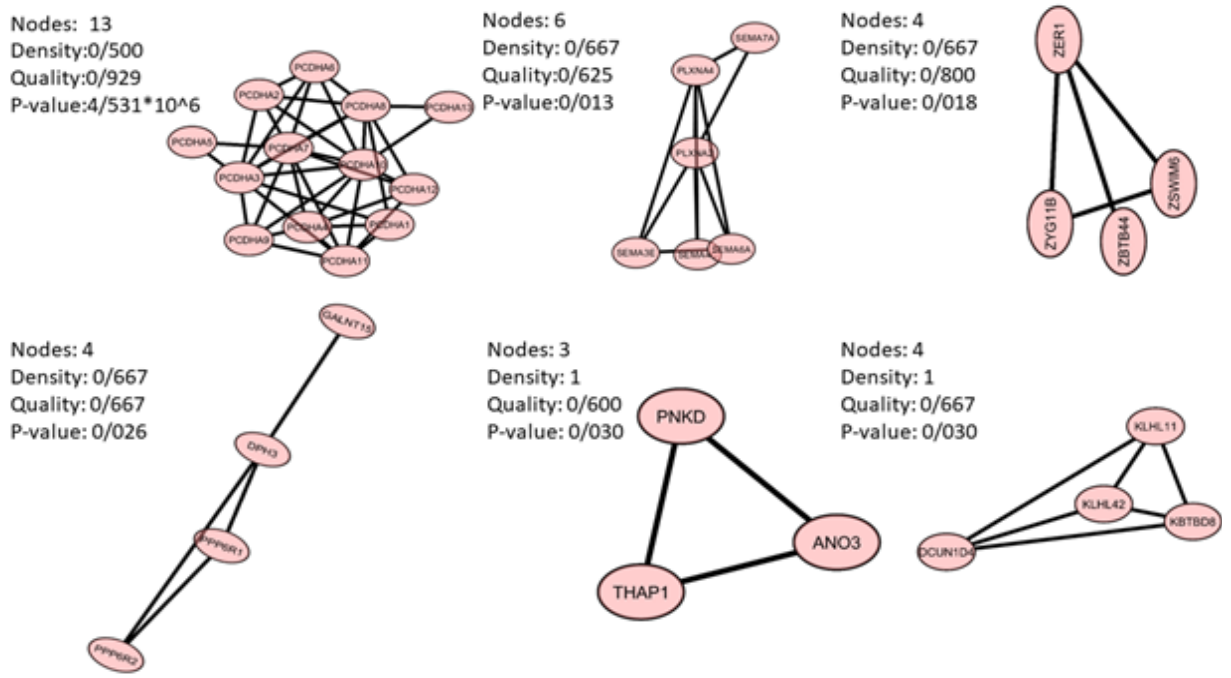

Figure 3. Drug Analysis of Hub Proteins

of targeted proteins with receptors involved in cellular homeostasis and tumor progression. The biological processes (BP) enriched included: Regulation of DNA-Templated Transcription (GO:0006355): A key process in gene expression control. Positive Regulation of Transcription by RNA Polymerase II: Emphasizing roles in active gene transcription. Regulation of Translation: Suggesting an influence on protein synthesis, critical in cancer progression.

The pathway enrichment analysis (WikiPathway) identified critical pathways, such as:

EGFR Signaling (WP437): A pathway heavily implicated in colorectal cancer progression and targeted therapies. DNA Damage Response and ATM-dependent pathways were also enriched, highlighting the involvement of these genes in genomic stability and repair mechanisms Figure 2.

Network Cluster Analysis of proteins

Using the CytoCluster plugin, six significant clusters were identified within the protein-protein interaction (PPI) network. Each cluster represents a highly interconnected submodule of proteins targeted by dysregulated miRNAs. The clusters exhibited varying node counts, densities, and P-values, indicating their statistical and biological significance (Figure 3). Cluster 1 (P-value: 4.531×10^{-6}): Involves members of the protocadherin family, associated with cell-cell adhesion and signaling, critical in cancer metastasis. Cluster 2 (P-value: 0.013): Highlights semaphorin signaling proteins, which regulate tumor cell migration and microenvironment interactions. Other clusters involve pathways related to transcriptional regulation, glycosylation, and protein ubiquitination. These clusters reveal functional modules that may play crucial roles in colorectal cancer progression and offer potential targets for further therapeutic investigation Figure 3.

Drug Analysis of hub proteins

The analysis of hub proteins identified through the PPI network revealed several drugs and compounds targeting these proteins. The DrugBank database provided

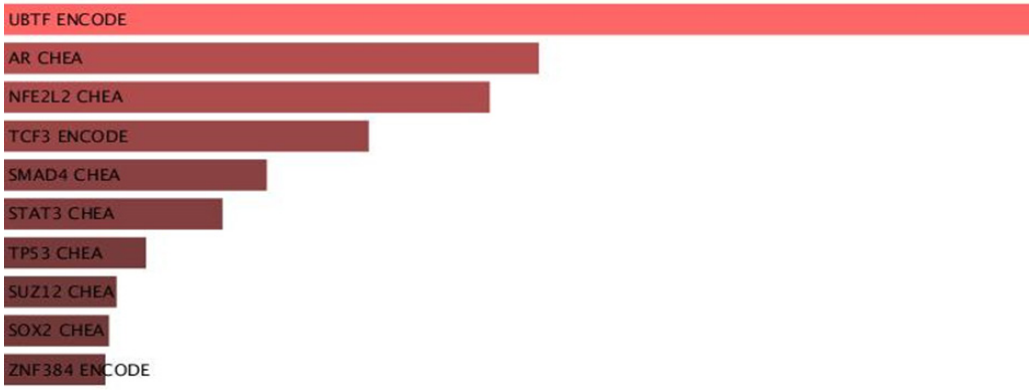

Figure 4. Key Transcription Factors were Identified Using Datasets from ENCODE and ChEA

Table 3. Drugs and Compounds Targeting Hub Proteins

Drug	Drug group	Pharmacological action?	Type	Actions	CREBBP
9-ACETYL-2,3,4,9-TETRAHYDRO-1H-CARBAZOL-1-ONE	experimental	unknown	target		CREBBP
Colforsin	experimental, investigational	unknown	target	activator	CREBBP
PRI-724	investigational	yes	target	modulator	CREBBP
Drug	Drug group	Pharmacological action?	Type	Actions	CREBBP
Conjugated estrogens	approved	yes	target	agonist	ESR1
Estrone	approved	yes	target	agonist	ESR1
Ethynodiol diacetate	approved	yes	target	agonist	ESR1
Ethinylestradiol	approved	yes	target	agonist	ESR1
Desogestrel	approved	yes	target	agonist	ESR1
Danazol	approved	yes	target	agonist	ESR1
Quinestrol	approved	yes	target	agonistmodulator	ESR1
Tamoxifen	approved	yes	target	antagonistagonist	ESR1
Mestranol	approved	yes	target	agonist	ESR1
Estrone sulfate	approved	yes	target	agonist	ESR1
Synthetic Conjugated Estrogens, A	approved	yes	target	ligand	ESR1
Synthetic Conjugated Estrogens, B	approved	yes	target	ligand	ESR1
Eugenol	approved	unknown	target		ESR1
Mitotane	approved	yes	target	binder	ESR1
Enzacamene	approved	unknown	target		ESR1
Norethynodrel	approved	unknown	target		ESR1
Dobutamine	approved	unknown	target		ESR1
Methyltestosterone	approved	unknown	target		ESR1
Testosterone cypionate	approved	unknown	target		ESR1
Testosterone enanthate	approved	unknown	target		ESR1
Polyestradiol phosphate	approved	yes	target	agonist	ESR1
Gestrinone	approved	yes	target	antagonistagonist	ESR1
Fluoroestradiol F-18	approved	unknown	target	binder	ESR1
Zinc sulfate, unspecified form	approved, experimental	unknown	target	binder	ESR1
Fluoxymesterone	approved, illicit	yes	target	antagonist	ESR1
Pilaralisib	investigational	unknown	target		PIK3CA
XL765	investigational	unknown	target		PIK3CA
Wortmannin	experimental	unknown	target		PIK3CA
Caffeine	approved	unknown	target	inhibitor	PIK3CA
ATP	investigational, nutraceutical	unknown	target		PIK3CA
Copanlisib	approved, investigational	yes	target	inhibitor	PIK3CA
Alpelisib	approved, investigational	yes	target	inhibitor	PIK3CA
CH-5132799	investigational	yes	target	inhibitor	PIK3CA
Serabelisib	investigational	yes	target	inhibitor	PIK3CA
LY-3023414	investigational	yes	target	modulator	PIK3CA
Taselisib	investigational	yes	target	modulator	PIK3CA
Buparlisib	investigational	yes	target	inhibitor	PIK3CA
Inavolisib	approved, investigational	yes	target	degradation	PIK3CA
Salirasib	investigational	yes	target	antagonist	NRAS
Adenosine phosphate	approved, investigational, nutraceutical, withdrawn	unknown	target	activator	CREB1
Naloxone	approved, vet_approved	no	target	other/unknown	CREB2

Table 4. Promoter Analysis of Hub Proteins

Motif	Logo	Length	Top 5 specific predictions
ENSG00000005339		19	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000011258		15	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000089818		18	MF olfactory receptor activity BP sensory perception of smell BP G-protein coupled receptor protein signaling pathway MF cytokine activity CC extracellular space
ENSG00000100393		20	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000170677		20	CC transcription factor complex BP negative regulation of signal transduction MF protein heterodimerization activity BP neuron fate commitment MF chromatin binding
ENSG00000213281		15	MF olfactory receptor activity BP sensory perception of smell BP G-protein coupled receptor protein signaling pathway BP regulation of immune response

a comprehensive list of therapeutic agents and their mechanisms of action. These findings highlight potential therapeutic avenues for colorectal cancer by targeting dysregulated pathways. For CREBBP (CREB Binding Protein), experimental and investigational agents such as PRI-724 (modulator) and Colforsin (activator) are under investigation for their roles in modulating CREBBP activity. Approved drugs, including conjugated estrogens, act as agonists, providing a therapeutic mechanism to regulate CREBBP. In the case of ESR1 (Estrogen Receptor 1), approved drugs such as Tamoxifen (antagonist/agonist) and various estrogens like Estrone and Ethinylestradiol act as agonists targeting ESR1. These compounds are significant for their dual roles in promoting or inhibiting receptor activity, making them valuable in hormone-driven cancer therapies. Eugenol and other compounds are being studied for their binding and regulatory roles. PIK3CA (Phosphatidylinositol-4, 5-Bisphosphate 3-Kinase Catalytic Subunit Alpha) has approved drugs such as Alpelisib and Copanlisib, demonstrating the potential to suppress aberrant PIK3CA signaling in cancer progression. Investigational compounds include agents such as Taselisib and Buparlisib, which are being explored for their inhibitory effects on PIK3CA, emphasizing the protein's therapeutic relevance. Compounds like Adenosine phosphate (activator) show potential for modulating NRAS (Neuroblastoma RAS Viral Oncogene Homolog) activity, although further studies are needed to establish therapeutic implications. The drugs and compounds identified target key hub proteins integral to colorectal cancer progression. Approved drugs, such as Tamoxifen and Alpelisib, highlight the potential for repurposing established therapies. Experimental agents like PRI-724 and investigational inhibitors of PIK3CA provide opportunities for novel therapeutic strategies. These findings underscore the importance of targeting dysregulated pathways in colorectal cancer and pave the way for preclinical and clinical investigations (Table 3).

Transcription Factor Perturbation and Promoter Analysis of Hub Genes

The transcription factor perturbation and promoter analysis revealed significant insights into the regulatory mechanisms influencing the expression of hub genes. Utilizing the Enrichr database, key transcription factors (TFs) and their potential impacts on gene regulation were identified. Additionally, motif analysis using Tomtom and GOMO provided detailed information on conserved sequences within promoter regions of hub genes.

Key transcription factors were identified using datasets from ENCODE and ChEA, as visualized in Figure 4. The most enriched transcription factor, UBTF (ENCODE), suggesting its pivotal role in chromatin organization and rRNA synthesis, potentially influencing tumor growth and proliferation [21]. The next TF factor, AR (CHEA), Known for its roles in cellular signaling and androgen response, its dysregulation could contribute to cancer progression [22]. NFE2L2 (CHEA) consider as a regulator

of oxidative stress response, often associated with cancer cell survival and chemoresistance [23]. TCF3 (ENCODE) is A critical regulator of Wnt signaling and cell fate decisions, emphasizing its role in colorectal cancer [24]. Other transcription factors such as SMAD4 (CHEA), STAT3 (CHEA), TP53 (CHEA), SUZ12 (CHEA), and SOX2 (CHEA) were also identified, highlighting their involvement in pathways related to cell proliferation, differentiation, and apoptosis [25]. These findings indicate a complex regulatory landscape where multiple transcription factors converge to modulate the expression of genes involved in colorectal cancer.

Promoter motif analysis revealed specific motifs associated with the hub genes, as summarized in Table 4: Motif ENSG00000005339 and ENSG00000011258: Enriched for functions such as chromatin binding, negative regulation of signal transduction, and neuron fate commitment, implicating these motifs in transcriptional regulation and cellular differentiation. Motif ENSG00000100393: Demonstrated significant involvement in protein heterodimerization activity, reflecting its potential role in chromatin remodeling and gene transcription. Motif ENSG00000213281: Highlighted for its role in immune response regulation and G-protein coupled receptor signaling, linking it to tumor-immune interactions. Motif ENSG00000089818: Identified as significant for olfactory receptor activity and extracellular signaling, potentially contributing to interactions within the tumor microenvironment.

Discussion

This study comprehensively explored the regulatory landscape of miRNAs, transcription factors, and hub proteins involved in colorectal cancer (CRC), particularly within the Wnt/β-catenin signaling pathway. The findings underscore the potential of multi-layered bioinformatics analyses to uncover critical therapeutic targets and elucidate mechanisms of CRC progression.

The identification of hub proteins such as EP300, NRAS, and NF1 reinforces their established importance in CRC. For example: EP300: Consistent with prior studies, EP300's role as a co-activator in transcriptional regulation is crucial for tumor suppressor function and chromatin remodeling [26]. Studies in hepatocellular carcinoma have similarly highlighted its role in regulating p53, suggesting its therapeutic potential in multiple cancers [27]. The role of NRAS mutations in CRC, particularly in metastatic settings, has been widely documented [28]. Recent studies indicate that NRAS mutations can drive therapy resistance through MAPK pathway hyperactivation, paralleling findings in colorectal cancer [29]. As a Ras regulator, NF1's loss has been linked to tumor growth in glioblastoma and CRC. This study's results further support NF1 as a central regulator of Ras-driven cancers [30].These findings are consistent with recent work that integrates PPI networks to identify therapeutic targets in CRC, such as the study by Mollanoori et al., which identified nearly similar hub proteins using network centrality measures [31]. The enrichment of pathways like EGFR signaling and DNA damage response aligns with established therapeutic targets in CRC. Notably: Monoclonal antibodies such as cetuximab have shown efficacy in CRC by targeting EGFR [32].

DNA damage response's role in CRC progression and resistance to chemotherapy is well-documented. The enrichment of ATM-dependent pathways corroborates findings by Pećina-Šlaus et al., who emphasized their importance in genomic stability [33]. The study also highlights pathways such as Focal Adhesion and PI3K/Akt/mTOR signaling, consistent with recent findings linking these pathways to CRC metastasis and therapy resistance.

In the next step we explored Transcription Factor Perturbation and Promoter Analysis, This study identified transcription factors such as UBTF, TCF3, and TP53 as key regulators in CRC. These findings are consistent with and extend the results of recent research: Emerging studies in glioblastoma and lung cancer have highlighted UBTF's role in rRNA transcription and chromatin organization, supporting its role in tumor cell proliferation [21]. As a critical regulator of the Wnt/β-catenin pathway, TCF3's role in CRC is well-established. This study aligns with research by Brown et al., which demonstrated that TCF factors are essential for Wnt-mediated transcription in CRC [34]. In addition, Mutations in TP53 are among the most frequent in CRC and are associated with chemotherapy resistance [35].

The promoter analysis revealed conserved motifs associated with chromatin binding and immune regulation. These findings align with recent work in CRC where similar motifs were linked to immune evasion and transcriptional dysregulation [36].

This study identified drugs targeting hub proteins, such as Tamoxifen for ESR1 and Alpelisib for PIK3CA. These findings are supported by recent studies. Although primarily used in hormone-driven cancers like breast cancer, studies have demonstrated its efficacy in inhibiting Wnt signaling in CRC [37]. Alpelisib, the PIK3CA inhibitor, has shown promise in CRC clinical trials [38], consistent with findings by Yang et al., who emphasized its role in targeting PI3K/Akt signaling [39]. The study also highlights investigational compounds such as PRI-724 (targeting CREBBP), which aligns with recent trials exploring its ability to disrupt Wnt/β-catenin signaling in advanced head and neck cancer [40].

Conclusion and Future Directions

This study not only confirms findings from prior research but also provides new insights into the regulatory mechanisms of CRC. By integrating multi-omics data, it highlights potential therapeutic targets and pathways for CRC. Future work should focus on validating these findings experimentally and exploring combination therapies targeting miRNA, hub proteins, and transcription factors to overcome therapy resistance and improve patient outcomes.

Author Contribution Statement

All authors contributed equally in this study.

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Data Availability Statement

The data that support the conclusions of this study can be made available by the corresponding author upon a reasonable request.

Competing Interests

The authors declare that they have no competing interests related to the content of this article.

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